



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Matthew Baker

Application No.: 09/736,632

Filed: December 14, 2000

For: **ISOLATION OF NUCLEIC ACIDS**

Examiner: William O. Sandals, PhD

Art Unit: 1636

RECEIVED

DECLARATION UNDER OCT 16 2003
37 C.F.R. § 1.132

TECH CENTER 1600/2900

DECLARATION OF GARRY HARPER, PhD

1. I, Garry Harper, am Head of Material Science at DNA Research Innovations Limited, an employee of the company. A copy of my curriculum vitae is attached.

2. In this declaration, I describe the measurement of the pKa of the ECTHAM cellulose prepared as set out in the declaration of Matthew Baker which is being submitted concurrently with this declaration. For comparison, the pKa of three other materials was also determined. The materials were two commercially available ion exchangers, DEAE Sepharose and DEAE Sephadex A50 (Sigma Chemical Co, St. Louis MO, USA. Lot Nos: 11K1329 and 10K0790 respectively) and a sample of Bis-Tris magnetic beads, a charge switch material conforming to the above mentioned patent application. These beads were 0.8 μ m beads (DRI Lot No. V2006) coated with poly Bis-Tris. The beads were made by mixing carboxylated magnetic beads with Bis-Tris and a coupling agent EDC as previously discussed in the patent application.

3. The pKa measurements were made using acid to base titration (HCl-NaOH, pH range 1-13) and by measuring the isoelectric point (iep) determined from zeta potential measurements using laser-doppler anemometry on a Malvern Zetasizer 3000HSA (Model DTS5301 Serial No: 34539/96 Malvern Instruments Ltd. Malvern WR14 1X2 UK). Titration was performed on Hydrochloric acid acidified samples from pH1 through pH13, using addition of NaOH 1M, to achieve discrete pH unit intervals of 1 (+/- 0.05) pH unit providing

13 points for curve fitting. pH measurements were performed using a Mettler Toledo MP220 (Serial No: 205649M, Mettler-Toledo, Schwerzenbach, Switzerland) fitted with a BDH Gelplas (BDH Poole, UK; Cat No: 309/1051/03) double junction combination electrode. The Zeta potential was recorded at each pH unit interval of the titration from measurements made on aliquots of the titration stock.

The pKa values were then determined from plots of Zeta potential (mV) against pH, by both inspection (pH when mV = 0, at the isoelectric point) and by using MacCurveFit software (Version 1.4, Kevin Raner Software 1998 on an Apple iBook running MacOS system 9.2), using the most appropriate (highest) r^2 fit to the whole dataset from three models: Sigmoid, Linear and 2nd Order Polynomial.

4. These fitted measurements gave the following results:

	pKa
ECTHAM cellulose	12.55
DEAE Sepharose	14.16
DEAE Sephadex A50	14.23
Bis-Tris charge switch beads	5.49

5. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Declarant's signature

Date:

22 September 2003

CURRICULUM VITAE

SEPTEMBER 2003

GARRY ROBERT HARPER, B.Sc.(Joint Hons.), Ph.D. (Cantab.)

Present position: Head of Material Science
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Current Research Area: Solid-phase development of DNA-binding polymers

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Sittingbourne
Kent ME10 1PP
United Kingdom
Tel: (44)-01795-436428
E-mail: GRH3@aol.com

Nationality: British/Canadian Dual National.
Birth: 29th December 1958, Hawkesbury Canada

Academic qualifications:
B.Sc. (Hons.), 2.1, Bristol University 1980.
Ph.D. (Applied Biology), Cambridge University 1987.

POSITIONS HELD:

2001-Present DNA Research Innovations Ltd, Sittingbourne Research Center, UK.
Senior Scientist responsible for solid-phase development utilising DNA-binding polymers.
Polyion patent submission 2002. Promoted to Head of Material Science- January 2003.

1998-2001 RAE Postdoctoral Research Fellow/ Laboratory Manager.
Biomaterials and Drug Delivery (BADD) Group, School of Pharmacy and Biomedical
Sciences, University of Portsmouth, Portsmouth, UK.

Research on steric stabilisation of polymeric nanoparticulate delivery systems that utilise covalently bound lectins (saccharide-binding proteins) as cell-surface targeting groups. School Radiation Protection Supervisor, PhD Supervisor (2 - Industry Sponsored). Guest lecturer in Organic Biochemistry (Year II Environmental Sciences - 10 lectures + Exam). Lectures in Statistics (6 lectures) to Biomedical Science MSc 2000.

1994-1997 Scientist and Science Area Manager.
Biochemical Science, Natural Products Processing Division, Industrial Research Limited
(Government Research Institute) , Wellington, New Zealand (Formerly DSIR).
Surface and biochemical separation science. 1996 Promoted grade 7 to 8.

1991-1993 Scientist - Biophysics. Surface Science Group,
Dept. Polymer Chemistry, Becton Dickinson Research Center, Davis Drive, Research
Triangle Park, NC, USA. Blood-polymer surface interactions

1989-1990 Postdoctoral Research Associate/Instructor.
Dept. Artificial Organs, Biomaterials and Cellular Technology, Division of Biology and
Medicine, Brown University, Providence, Rhode Island, USA. Polymeric cell encapsulation
technologies for single-factor deficiency therapies for Parkinsonism & Diabetes.

1986-1989 ICI-SERC Postdoctoral Research Fellow
Dept. Pharmaceutical Sciences, Nottingham University, UK.
Sterically-stabilised polymeric microsphere drug delivery systems.

1980-1985 Postgraduate Research Student.
Post-Harvest Unit, Dept. Applied Biology, Cambridge University, UK. 'Structure and
permeance of polymeric coatings on fruit' - TAL Prolong™
SERC-CASE Award Geest Holdings, Tate & Lyle and the Wolfson Foundation.

DETAIL OF INDUSTRIAL RESEARCH EXPERIENCE:

1994-1997 Scientist & Science Manager - CRI Industrial Research Ltd, New Zealand.
Research scientist and research manager (promoted Grade 7 to 8, 1996) with industrial
separation technologies programme within biochemical processing science team, with
particular reference to:

- 1.- Novel biomaterials and charged polymer films for bioedible and biotechnology coatings,
- 2.- Colloid and surface science applied to biological interactions at polymer surfaces,
- 3.- Development of charge related separation processes using AEA technology.

Experienced in bidding for funding (NZ, USA & UK) and technology transfer, majority of
research has been confidential (contract, consultancy and collaborative).

- Successfully co-bid Industrial Separation Technologies Programme (1994: £400k/year
funding for 4 years) containing 6 novel biochemical separation projects.
- Managed science area of colloid and interface science implemented for biochemical
separations, including Light Scattering Spectroscopy (PCS) and Electrokinetics (Malvern
ZetaMaster).
- Obtained multi-year funding for collaborative research on conducting polymer colloids for
biochemical separations, and biofunctional edible films (£15,000 each for two years).

- Active collaboration with five departments in four Universities (Wollongong, Victoria, Massey & Dunedin (Pharmacy)) in areas of polymer colloids and charged membrane separations.
- Supported and maintained BPS Team ISO9000 accreditation - BPS TQM Chair 1995-1996.

1990-1993. Scientist - Becton Dickinson Research Center, RTP, NC, USA.

- Established a new laboratory (c \$300,000) with advanced colloid & biomaterials capability (Brookhaven Photon Correlation Spectroscopy, PenKem electrokinetics, protein assays & kinetics, protein separation and microscopy, [group possessed advanced surface analysis XPS and SIMS]).
- Related systematically modified polymer surfaces and their surface characteristics to initiate blood clotting and complement cascades, control adhesion of fibrin and blood clots for product applications.
- Supported US\$200 million/year business sector with 5 U.S. patents issued & implemented as Vacutainer™ PLUS tube series, with total of about 28 worldwide patents issued.

DETAIL OF ACADEMIC RESEARCH EXPERIENCE:

1987-2001 Postdoctoral Research Fellow, Biomaterials and Drug Delivery Group,
School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK.

- Research project on Lectin-polymer conjugates for ocular drug delivery.
- Laboratory Manager Biomaterials and Drug Delivery [BADD] Group
- PhD Research CoSupervisor for 2 Postgraduate students, sponsored by Pfizer UK and SmithKline Beecham (now GSK).
- Radiation Protection Supervisor School of Pharmacy & Biomed 1987-2001.
- 1999-2000 10 Lectures and examinations set in Organic Biochemistry to 2nd Year B.Sc.Environmental Science undergraduates.
- 2000 Lectures in Statistics to Biomedical Science MSc Course.

1989-1990 Instructor/Postdoctoral Research Associate, Brown University, USA.

Research interests:

- Collaborated with Cellular Transplants Inc. on polymeric viable cell encapsulation.
- Polymer Colloids for Drug Delivery (Burroughs Wellcome Appl.1 Student).
- Physico-chemical characterization of polyelectrolyte complexes (2 Students).
- Biopharmaceutics: Methods for therapeutic cell encapsulation.
- Sterically stabilised copolymers for biocompatible cell encapsulation.
- Established colloid and surface analysis laboratory capability (Cahn Dynamic Contact Angle & Parr Solution Calorimeter c.£80,000 Capex).1986-1989

ICI-SERC Research Fellow. Department of Pharmaceutical Sciences, Nottingham University, University Park, Nottingham UK.

Project: 'Sterically-stabilised colloidal particles as drug-delivery systems',

Seconded to two ICI (Agrochemical & Pharmaceutical) divisions.

- Synthesis of chemically defined copolymer microspheres with surfaces stabilised against surface adhesion by a steric barrier of graft poly(ethylene oxide) (at former ICI Agrochemicals).
- Physico-chemical characterization used to assess the effect of steric barrier thickness, molecular weight, electrophoretic mobility, size and hydrophobicity on the stability of these potential drug carriers for blood compartment delivery.
- Investigated extent to which uptake of these microspheres by macrophages of the reticuloendothelial system (RES) could be modified *in vitro* (primary cultures) and *in vivo* (radiolabelled), in order to direct the particles to different organ and tissue sites of the body (at former ICI Pharmaceuticals),

1980-1985 Research Student. Dept. Applied Biology, Cambridge University. UK.

Ph.D. Thesis: 'Structure and permeance of coatings on fruit'.

- Research on effects of a post-harvest coating, TAL-Prolong™ on gas exchange properties of banana fruit. Including physico-chemical investigation of the coating formulation of constituent polyanion with mixed surfactant systems. Supervisor: Prof. Sir James Beament FRS.

PUBLICATIONS:

1. Barisci, J.N., Hodgson AJ, Liu L, Wallace GG and Harper G. (1999) Electrochemical production of protein-containing polypyrrole colloids. *Reactive and Functional Polymers* 39(3): 269-275.
2. Beaglehole, D., Lawson, F. , Harper G. & Hossain, M. (1997) 'Tryptophan, tryptophan-leucine and BSA adsorption at a decanol oil-water interface.' *Journal Colloid and Interface Science* 192 p266-268.
3. Aboutanos V., Wallace G.G. & Harper G. ' Electrochemical Production of Polypyrrole Colloids containing Lactoferrin' 10th Australian Conference on Colloids and Surfaces Queensland 30 Sept. -3 Oct. 97.
4. Vogler E.A., Graper J.C, Harper G.R, Sugg H, Lander L., & Brittain W, (1995)'Contact activation of plasma coagulation cascade I, Procoagulant surface chemistry and energy.' 1. *Biomedical Materials Research* 29 p1005-1016.
5. Harper G.R., Nijdam J. & Stanley R (1995) 'Selective retention of a sodium caseinate dispersion using an electrically charged membrane system.' *Proceedings 23rd Australasian Chemical Engineering Conference* 3:56-61.

PUBLICATIONS continued:

6. Harper, G.R., Davis, S.S., Davies, M.C., Norman, M.E., Tadros, T.F., Taylor D.C., Irving, M.P., Waters, J.A. & Watts, J. (1995) 'Influence of surface coverage by Poly(ethylene oxide) on attachment of sterically stabilised microspheres to Rat Kupffer cells *in vitro*.
Biomaterials 16 p427-439.
7. Davis S.S., Illum, L., Washington, C. & Harper, G. (1992) 'Studies on the interaction of charge-reversed emulsions with the reticuloendothelial system.'
Internat. J. Pharmaceutics. 82 p99-105.
8. Harper, G.R., Davies, M.C., Davis, S.S., Tadros, T.F., Taylor, D.C., Irving, M.P. & Waters, J.A (1991) 'Steric stabilisation of microspheres with grafted Poly(ethylene oxide) reduces phagocytosis by rat Kupffer cells *in vitro*.'
Biomaterials 12 p675-700.
9. Davis S.S., Illum, L., Muller, R., Landry, F., Wright, J. & Harper G. (1990) 'Effect of infused fat emulsions on reticuloendothelial function in the rabbit'. Clinical Nutrition (Edinb.) 9(5) p260-265.
10. Norman, M.E., Harper, G.R., Williams, P.A. & Illum L. (1989) 'Opsonisation of sterically stabilised colloidal carriers and their interaction *in vitro* with Kupffer cells.' J.Pharm. Pharmacol. (1989) 41 (Suppl.) 127P.
11. Young, Y.Y., Harper, G.R., Illum, L., & Davis, S.S. (1989) 'Separation of biomodal particle suspensions using counterflow centrifugation.' Pharm. Pharmacol. 41 (Suppl.) 126P.
12. G.R. Harper G., J. Smith, J. Tsibouklis and J. Smart. Physico-chemical study of polymeric microparticles bearing Concanavalin-A and sterically stabilised by Methoxy-poly(ethylene oxide).
Proceedings InterLec18 - 18th International Lectin Conference, Portsmouth, UK August 1999.

PATENTS:**2003 DNA Research Innovations submission:**

'Materials and Substances Relating to Polyions and Substance Delivery'. Inventors: Matthew Baker, Garry Robert Harper & Paula Cooper . PCT Application PCT/GB2003/002417 Priority 31/05/02.

US Patents Issued:

Becton Dickinson Assigned Patents (5 Core USPTO - 28 Worldwide Issues):

- 1 US Patent Serial No. 5,320,812. Issued June 14, 1994. 'Clot Activating Polyelectrolyte Complex and Blood Collection Assembly Containing Same'. Harper G.R.
- 2 US Patent Serial No. 5,565,611 Issued Sept. 6, 1994, 'Vacuum Actuated Blood Collection Assembly Including Tube of Clot-Accelerating Plastic.' Vogler E.A. & Harper G.R.,
- 3 US Patent Serial No. 5,326,535 Issued July 5, 1994, 'Tube having Unitary Blood Coagulation Activator and Method for its Preparation.' Vogler E.A., Graper J. & Harper G.R.

- 4 US Patent Serial No. 5,257,633, Issued Nov. 2, 1993 (& Europe Pat.No. EP576184) 'Surface Modified Blood Collection Tubes' Vogler E.A.& Harper G.R.
- 5 US Patent Serial No 5,256,666. Issued Sept. 10, 1993 (& EPO). 'Additive having dual surface chemistry for blood collection container & assembly containing same.' Vogler E.A. & Harper G.R.

RESEARCH GRANT APPLICATIONS:

1994/1995: • Industrial Separation Technologies Programme rebid.

Awarded (£1,600,000 over 4 Years). Sponsor: CRI Industrial Research,NZ.

- NSOF Funding for Conducting Polymer Colloids (£10,000x 2 years).
- FoRST Funding for Colostrum Separation Technology - Awarded.
- NSOF Funding for Bioactive Post-Harvest Coatings (2 year seed funding).

1993: • Wellcome Foundation. Drug development- Cell Contact Phenomena.

Sponsor - Department of Pharmaceutical Sciences. Strathclyde Univ. Not funded.

1990: • Full Invited Application 'Development of novel polymeric cell

encapsulation by photo-polymerization'. Whittaker Foundation: (£112,000)

Not Funded. Sponsor: Brown University, USA.

1990: • Full Invited Application Burroughs-Wellcome Fund.

Drug-delivery (£200,000 over 3 years), Not funded. Sponsor: Brown University, USA.

1985 • NERC Postdoctoral Fellowship Application Submitted -

Non-UV 'Deactivation of the Baculovirus of Budworm (Spodoptera littoralis) on Cotton (Gossypium hirsutum) leaf surfaces.'

Sponsor- NERC Institute of Virology Oxford. NERC Alpha-rated (i.e. Awarded),

Not Funded due to Natural Environment Research Council (NERC) bankruptcy 1985.

COURSES ATTENDED:

- 1994 • 'Molecular Biology Methods for the Identification of Micro-organisms' Mohawk College, Ont. Jan- Mar. Accredited Module.
- 1988 • 'Radioisotopes Techniques Short Course', Loughborough University. June 21-July 1.
- 1986 • 'Solid/Liquid Dispersions' Royal Society of Chemistry (RSC) Bristol University, 19-25th July.
- 1986 • 'Technologically important aspects of interface science', Interface Science Group, Imperial College 19th September.
- 1981 • Biometry - Experimental Design and Statistics M.Sc. Course. Lectures, manual & GENSTAT practicals required attendance as postgraduate student. Dept. Applied Biology, Cambridge University.

MEETINGS ATTENDED [* Asterisk denotes Presentation]:

- 2003 • Controlled Release Society (CRS) Annual Meeting 2003 Glasgow, UK July 2003**
- 2002 • Medica Trade Meeting, Dusseldorf, DE October.
- 1999 • Controlled Release Conference 1999 UKCRS, Aston University, Birmingham, UK.
- 1998* • NewPharm-98. National Pharmaceuticals Research Conference, Portsmouth, UK.
- 1998 • Controlled Release Seminar, Department of Trade & Industry, Westminster, London.
- 1997 • International Australasian Biosensors Meeting, Wellington, New Zealand, Jan.
- 1996* • Intelligent and Advanced Polymers Conference - Intelligent Polymer Research Lab., University of Wollongong, NSW, Australia. Dec.
- 1995* • CHEMECA Annual Chemical Engineering Conference, Adelaide, SA, Australia August.
- 1995 • Intelligent Polymers Workshop - Intelligent Polymer Research Lab., University of Wollongong, NSW, Australia. Feb.
- 1994 • International Australasian Biosensors Meeting. Wellington, NZ Nov.
- 1993 • Society for Biomaterials, 202nd Meet. Birmingham Alabama, USA May.
- 1992 • 'Characterization of Biomaterial Surfaces', CMSA, Manchester, UK May 18-20.
- 1991 • Society for Biomaterials, 200th Meet. Phoenix AZ, USA May 1-5.
- 1990 • American Chemical Society, 199th Meet., Boston, MA USA April 23-27.
- 1990 • Society for Biomaterials, 199th Meet. Charleston SC, USA May 19-23.
- 1990 • 64th ACS Colloid & Interface Science Meet. Lehigh PA, USA June.
- 1990 • 'Biointeractions', Butterworths Biomaterials Conference, Oxford. Aug. 21-23.
- 1989 • 'Microbial Inoculants in Agriculture', SCI, London, February 14.
- 1989 • British Pharmaceutical Conference (BPC), September, Keele.
- 1988 • 'Particle Deposition at the Solid/Liquid Interface'. University College, London Sept.
- 1988 • 'Polymers for Life Science applications', Macro Group SCI London, December 7.
- 1988 • 'Preparation of Colloidal Particulates', Society of Chemical Industry, London.
- 1987 • 'Colloidal Aspects of Pharmaceutical and Pesticide Formulation' SCI.
- 1986 • 'Colloid Science in Biology', Society of Chemical Industry (SCI), London.
- 1981* • British Association Advancement Science, York 50th Meeting.

INVITED ATTENDANCE & SEMINARS GIVEN:

- 1999 • 'Steric Stabilisation & Cell Contact' School Pharmacy, Portsmouth, Seminar Series.
- 1998 • 'Steric Stabilisation & Cell Contact' Seminar presentations NewPharm & University Seminar Series.
- 1997 • 'Steric stabilization of Polymer Microspheres and its role in Cell Contact.' Biotechnology Centre, Cranfield University, Beds, UK.
- • 'Steric-stabilisation and cell-contact for site specific delivery' ISIS Pharmaceuticals, San Diego, CA, USA, Aug.
- 1996 • 'Cell-contact phenomena using sterically-stabilised polymer colloids' Intelligent and Advanced Polymers Conference- Intelligent Polymer Research Lab.,

University of Wollongong, NSW, Australia. Dec.

- 1994 • 'Post-harvest coatings and plant surface characterisation'
Dept. Plant Sciences, Massey University, Palmerston North, NZ.
- 1993 • 'Intracellular Targeting and Cell Containment for Therapeutic Delivery' Ribozyme
Pharmaceuticals, Boulder, Colorado, USA May.
- 1990 • 'Cell Encapsulation Technology' Somatix Corporation, Cambridge MA August.
- 1990 • 'Aspects of Cell encapsulation Technology' Cell Biotech Corp.(Transcel).
Century City, Los Angeles, CA July.
- 1990 • 'Colloidal Delivery Systems', Becton Dickinson Research Center, RTP, NC October.
- 1989 • 'Drug Targeting using Colloidal Delivery Systems', Artificial Organ
Laboratory, Brown University, August.
- • 'Steric Stabilisation' Shell Research Center, Sittingbourne, Kent, August.
- 1988 • AFRC Workshop on 'Release of Genetically Engineered Organisms (GMOs) into the
Environment' Heathrow, London. October 20.
- 1984 • 'Characterization of Plant Surfaces'. NERC Institute of Virology.

MEMBERSHIPS OF SOCIETIES:

American Chemical Society (1990-91,93),
Society for Chemical Industry, UK (1986-1990)

MISCELLANEOUS:

Full Driving Licences - United Kingdom, United States, Canada & New Zealand